

AD _____

Award Number: DAMD17-01-1-0511

TITLE: Development of an EBV-Mammary Epithelial Cell Model for
Addressing the Role of EBV in Breast Cancer

PRINCIPAL INVESTIGATOR: Erik K. Flemington, Ph.D.

CONTRACTING ORGANIZATION: Tulane University Health Science Center
New Orleans, Louisiana 70112

REPORT DATE: August 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030114 034

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE August 2002	3. REPORT TYPE AND DATES COVERED Annual (1 Aug 01 - 31 Jul 02)	
4. TITLE AND SUBTITLE Development of an EBV-Mammary Epithelial Cell Model for Addressing the Role of EBV in Breast Cancer			5. FUNDING NUMBERS DAMD17-01-1-0511	
6. AUTHOR(S) Erik K. Flemington, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Tulane University Health Science Center New Orleans, Louisiana 70112 E-Mail: eflemin@tulane.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) In this grant we proposed to generate an EBV infected mammary epithelial system that could be used to characterize the life cycle and oncogenic properties of EBV in this unique tissue. We have been utilizing primary human mammary epithelial cell lines generated in Myles Brown's laboratory. By Fluorescence Activated Cell Sorting (FACS), we determined that these cells are positive for the EBV receptor, CR2 and we have been carrying out infection studies to determine the infectability of these cells to EBV. To date we have found that despite the presence of the CR2 receptor, infectability is relatively low. This makes transient studies more difficult. We have therefore, begun generating long term cultures of infected IMEC cells through a variety of different means. We believe that we are making encouraging progress but we estimate that the completion of this project will require another year to complete. We have therefore obtained a 1 yr no cost extension to allow us to complete this work.				
14. SUBJECT TERMS breast cancer, Epstein Barr virus			15. NUMBER OF PAGES 6	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	4
Reportable Outcomes.....	4
Conclusions.....	5
References.....	5
Appendices.....	

INTRODUCTION:

EBV is a human herpes virus that infects B-lymphocytes as well as epithelial cells (9, 16, 18). EBV is the causal agent of infectious mononucleosis and is associated with the development of both B-cell and epithelial cell malignancies including the endemic form of Burkitt's lymphoma, post-transplantation lympho-proliferative diseases, AIDS-associated lymphomas, Hodgkin's disease, undifferentiated nasopharyngeal carcinoma (8, 13). EBV has also been linked to some cases of gastric carcinoma (2, 7, 17), T-cell lymphoma (15), and lymphoepithelioma-like carcinoma in the salivary glands, lung, and thymus (4, 14, 19). Although the possible role of EBV in breast cancer has been controversial, more recent publications have provided a potentially interesting correlation between EBV and breast cancer (1, 3, 5, 6, 10-12). This led us to propose investigating this relationship further. We proposed to generate a tissue culture model in which the biology of EBV in mammary tissues could be studied as well as its possible role in the initiation and/or maintenance of breast cancers.

BODY:

Since we found that human immortalized mammary epithelial cells (IMECs) express the CR2 receptor, we believed that we could infect these cells with EBV to address EBV biology and EBV's influence on cell growth/transformation properties. Nevertheless, based on previous work in our lab as well as many other labs studying EBV, it is clear that EBV infection is typically low level despite the presence of CR2 on target cells. We have now addressed the efficiency of EBV infection in IMEC cells. For our initial studies, we generated virus from the B95-8 cell line after several days and high density. Using these conditions, we obtained <1% of cells infected with EBV as measured by immunofluorescence against EBNA1. In our application, we proposed to address the pattern of EBV gene expression during the initial stages of viral infection. Since these studies could be more readily carried out if we were able to obtain higher levels of infection, we carried out further experiments in an effort to obtain a higher infection efficiency. We tested whether concentration of virus generated from B95-8 cells yields higher infection efficiencies but infection efficiencies remained low. We therefore tested an alternative (although more expensive) method in an effort to obtain higher titers of EBV. Treatment of the EBV positive Burkitt's lymphoma cell line, Akata, with anti-Ig yielded significantly higher infection efficiency and we believe that these conditions will be sufficient to be able to carry out informative EBV gene expression studies.

We have also begun soft agar assays following infection to address whether EBV elicits any transformed phenotypes in IMECs. Although preliminary, our first set of experiments suggest that EBV indeed yields a substantially higher number of colonies in soft agar relative to control treated cells. If this result repeats, this is important not only from a conceptual standpoint but this will also be an effective means for us to select long term EBV expressing IMEC clones. This will allow us to address more of the EBV biology and gene expression patterns as well as cell cycle alterations that are conferred by EBV.

KEY RESEARCH ACCOMPLISHMENTS:

- Determined that IMEC cells express the EBV receptor, CR2.
- Identified infection conditions that are suitable for carrying out studies addressing the EBV gene expression profiles during initial infection.
- Obtained preliminary evidence that EBV enhances growth in soft agar.
- Begun the generation of long term IMEC cultures containing EBV.

REPORTABLE OUTCOMES:

- We are in the process expanding colonies from cultures infected with EBV. Pending characterization and cryopreservation, these cultures will be made available to other investigators.

CONCLUSIONS:

- 1) We have identified infection conditions that are suitable for characterizing the EBV gene expression program during initial infection. In addition, we have preliminary evidence that EBV enhances growth in soft agar. This will allow us to generate cell clones expressing EBV and leads investigations towards a possible role of EBV in the progression of breast cancer.

REFERENCES:

1. Bonnet, M., J.-M. Guinebretiere, E. Kremmer, V. Grunewald, E. Benhamou, G. Contesso, and I. Joab. 1999. Detection of Epstein-Barr virus in invasive breast cancers. *J. Natl. Cancer Inst* 91:1376-1381.
2. Burke, A., T. Yen, K. Shekitka, and L. Sobin. 1990. Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. *Mod Pathol* 3:377-380.
3. Chu, J., C. Chen, and K. Chang. 1998. In situ detection of Epstein Barr virus in breast cancer. *Cancer Lett* 124:53-57.
4. Dimery, I., J. Lee, M. Blick, G. Pearson, G. Spitzer, and W. Hong. 1988. Association of the Epstein-Barr virus with lymphoepithelioma of the thymus. *Cancer* 61:2475-2480.
5. Gaffey, M., H. Frierson, S. Mills, J. Boyd, R. Zarbo, J. Simpson, L. Gross, and L. Weiss. 1993. Medullary carcinoma of the breast. Identification of lymphocyte sub-populations and their significance. *Mod Pathol* 6:721-728.
6. Glaser, S., R. Ambinder, J. DiGiuseppe, P. Horn-Ross, and J. Hsu. 1998. Absence of Epstein Barr virus EBER-1 transcripts in an epidemiologically diverse group of breast cancers. *Int J Cancer* 75:555-558.
7. Imai, S., S. Koizumi, M. Sugiura, M. Tokunaga, Y. Uemura, N. Yamamoto, S. Tanaka, E. Sato, and T. Osato. 1994. Gastric carcinoma: monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. *Proc Natl Acad Sci USA* 91:9131-9135.
8. Kieff, E. 1996. Epstein-Barr virus and its replication, p. 2343-2396. *In* B. C. Fields, D. M. Knipe, and P. M. Howley (ed.), *Fields Virology*, Third ed. Lippincott-Raven Press, Philadelphia.
9. Kieff, E., and D. Liebowitz. 1990. Epstein-Barr virus and its replication., p. 1889-1920. *In* B. N. Fields, and D. M. Knipe (ed.), *Virology*. Raven Press, New York.
10. Labrecque, L., D. Barnes, I. Fentiman, and B. Griffin. 1995. Epstein-Barr viruses in epithelial cell tumors: a breast cancer study. *Cancer Res* 55:39-45.
11. Lespagnard, L., P. Cochaux, D. Larsimont, M. Degeyter, T. Velu, and R. Heimann. 1995. Absence of Epstein-Barr virus in medullary carcinoma of the breast as demonstrated by immunophenotyping, in situ hybridization and polymerase chain reaction. *Am J Clin Pathol* 103:449-452.
12. Luqmani, Y., and S. Shousha. 1995. Presence of Epstein-Barr virus in breast carcinoma. *Int J Oncol* 6:899-903.
13. Miller, G. 1990. Epstein-Barr virus., p. 1921-1958. *In* B. N. Fields, and D. M. Knipe (ed.), *Virology*. Raven Press, New York.
14. Raab-Traub, N., P. Rajadurai, K. Flynn, and A. Lanier. 1991. Epstein-Barr virus infection in carcinoma of the salivary gland. *J Virol* 65:7032-7036.
15. Rickinson, A., and E. Kieff. 1996. Epstein-Barr virus, p. 2397-2446. *In* B. Fields, D. Knipe, and P. Howley (ed.), *Fields Virology*, Third ed. Lippincott-Raven Publishers, Philadelphia.
16. Rickinson, A. B. 1986. Cellular immunological responses to infection by the virus., p. 75-125. *In* M. A. Epstein, and B. G. Achong (ed.), *The Epstein-Barr virus: recent advances*. William Heinemann, London.
17. Shibata, D., M. Tokunaga, Y. Uemura, E. Sato, S. Tanaka, and L. Weiss. 1991. Association of Epstein-Barr virus with undifferentiated gastric carcinomas with intense lymphoid infiltration. Lymphoepithelioma-like carcinoma. *Am J. Pathol* 139:469-474.
18. Sixbey, J. W., S. M. Lemon, and J. S. Pagano. 1986. A second site for Epstein-Barr virus shedding: the uterine cervix. *lancet* ii:1122-1124.

19. Wong, M., L. Chung, S. Yuen, S. Chan, E. Wang, and K. Fu. 1995. In situ detection of Epstein-Barr virus in non-small cell lung carcinomas. *J Pathol* 177:233-240.